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On the Holistic Approach in Cellular and Cancer Biology: Nonlinearity, Complexity, and Quasi-Determinism of the Dynamic Cellular Network

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A keystone of the molecular reductionist approach to cellular biology is a specific deductive strategy relating genotype to phenotype—two distinct categories. This relationship is based on the assumption that the intermediary cellular network of actively transcribed genes and their regulatory elements is deterministic (i.e., a link between expression of a gene and a phenotypic trait can always be identified, and evolution of the network in time is predetermined). However, experimental data suggest that the relationship between genotype and phenotype is nonbijective (i.e., a gene can contribute to the emergence of more than just one phenotypic trait or a phenotypic trait can be determined by expression of several genes). This implies nonlinearity (i.e., lack of the proportional relationship between input and the outcome), complexity (i.e. emergence of the hierarchical network of multiple cross-interacting elements that is sensitive to initial conditions, possesses multiple equilibria, organizes spontaneously into different morphological patterns, and is controled in dispersed rather than centralized manner), and quasi-determinism (i.e., coexistence of deterministic and nondeterministic events) of the network. Nonlinearity within the space of the cellular molecular events underlies the existence of a fractal structure within a number of metabolic processes, and patterns of tissue growth, which is measured experimentally as a fractal dimension. Because of its complexity, the same phenotype can be associated with a number of alternative sequences of cellular events. Moreover, the primary cause initiating phenotypic evolution of cells such as malignant transformation can be favored probabilistically, but not identified unequivocally. Thermodynamic fluctuations of energy rather than gene mutations, the material traits of the fluctuations alter both the molecular and informational structure of the network. Then, the interplay between deterministic chaos, complexity, self-organization, and natural selection drives formation of malignant phenotype. This concept offers a novel perspective for investigation of tumorigenesis without invalidating current molecular findings. The essay integrates the ideas of the sciences of complexity in a biological context.
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KEY WORDS: **complexity; fractals; nonlinearity; reductionism; genotype; phenotype; cancer**

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INTRODUCTION

This essay deals with the issue of phenotype formation from the holistic perspective of the sciences of complexity [1]. More specifically, it investigates the relationship between genotype and phenotype using ideas of nonlinear physics; nonlinearity (i.e., lack of the proportional relationship between input and the outcome), complexity (i.e., emergence of the hierarchical network of multiple cross-interacting elements which is sensitive to initial conditions, possesses multiple equilibria, organizes spontaneously into different morphological patterns, and is controled in dispersed rather than centralized manner), and quasi-determinism (i.e., coexistence of deterministic and nondeterministic events), and points out some properties of cells accounting for those specific features. The essay attempts to present also theoretical and practical consequences of those ideas for investigation of tumorigenesis. It suggests that a novel interpretation of tumorigenesis-related events, alternative to the genetic model, is possible without invalidating current molecular findings. It introduces fractal dimension as a parameter that can become useful in the evaluation of tumor differentiation and organization or dynamics of tumor progression. Finally, it emphasizes a significance of probability in cellular phenomena influencing deterministic interpretation of genetic assays such as gene overexpression or knock-out, as well as clinical prognosis and outcome.

There are a number of reasons that justify an holistic approach to cellular biology. First, cellular phenomena follow the logic of the natural laws; however, cells also exhibit unique features that cannot be explained as direct consequences of those laws and that seem to reflect advanced organization of eucaryotic cells [2]. Indeed, a normal living cell can be defined as a self-organizing, self-regulating, self-replicating, catalytic, nonlinear, complex, thermodynamic (i.e., isothermal and open), and molecular system operating on the principle of complementarity with a number of the interconnected variables, (i.e., factors and parameters), where both the intermediary cellular network of genes and their regulatory elements as well as phenotype are of dynamic nature [2–11]. Second, the Darwinian thinking that established a teleological dogma of the final cause of structure and function in classical biology (i.e., a dogma of cause based on purpose or design) and emphasized a role of natural selection in a continous evolution seems to be unable to predict the evolution of the supramolecular system such as a cell. Yet, teleology cannot easily be eliminated from the analysis of biological complexity at the cellular level because cells do possess goal-oriented structure. For example, periodic ion transfer and polarization of membranes underlie interactions between myosin, actin, and other related proteins resulting in myocyte contraction. Epithelial cells possess a number of ultrastructural and molecular elements facilitating secretion, absorption, or permeability barrier formation, and so forth [4]. Third, causality (i.e., the existence of the identifiable link between a cause and effect) is typical of the deterministic and linear systems. A belief that such a link can always be identified for any natural phenomenon resulted in determinism in physics, and has indirectly influenced cellular biology. However, this traditional objective of the natural sciences to relate cause and effect in a predictable manner cannot be achieved in the case of a complex system because deterministic chaos may coexist with complexity within such system [6,12]. Owing to the interconnectedness (i.e., existence of multiple links between elements of the system), neither the cause driving evolution of the whole hierarchical system can be identified unequivocally nor participation of the other elements of the system can be determined. Finally, the C-theory (i.e., the theory of complexity in nonlinear dynamic systems) is a set of concepts, ideas, models, and observations rather than a comprehensive theory. Therefore, a discussion about those ideas in the biological context can help to set up a link between physical and biological complexity [3,13].

The extensive research during the last several years has elucidated, at least in part, molecular events underlying the basic functions of eucaryotic cells such as proliferation, differentiation, growth, apoptosis, and phenotype formation [2,4,5]. Nonetheless, a relationship between genotype, defined as a reflection of the actual information present in the DNA at a particular locus, and phenotype, an observed biochemical or morphological characteristic [14] remains a central issue in both cellular and cancer biology [15]. A keystone of the molecular reductionist approach to the relationship between these two distinct categories is a specific deductive strategy. This strategy assumes that molecular elements of cells obey the laws of deterministic physics and chemistry [2,4,5], that a combination of those elements produces a molecular representation of a cell [2,5,16–18], and that biologically-relevant information is transfered in one direction only: from genes through mRNA to proteins [2,4,5]. This representation is deterministic because the relationship between gene expression and phenotypic trait can always be identified [2,5,19,20]. This principle is known as causality. The deductive strategy works within the frame of mendelian genetics, which requires that two of the four cells arising as a result of meiosis receive a gene from one of the haploid parent cells and that the other two cells possess the gene of the second haploid cell. Then, a role of the gene in the emergence of a phenotypic trait can be deduced from analysis of the mutated phenotype [2,14]. The mendelian relationship between genotype and phenotype possesses two features. First, a given set of the actively transcribed genes corresponds to a set of phenotypic traits. Second, each gene

contributes to the emergence of a single phenotypic trait and, inversely, each phenotypic trait is determined by expression of a corresponding gene. This type of the relationship is defined in the mathematical set theory as bijective (i.e., consisting of both surjective (the first feature) and injective (the second feature) part). The existence of the bijective relationship is a prerequsite condition for the cellular network to be deterministic and linear because only then genes and phenotypic traits could be related to each other unequivocally. In other words, the emergence of a phenotypic trait at the current time point would be determined by a pattern of gene expression at the previous time point. However, the deductive strategy is useless in case of a multifactorial and polygenic disease such as a sporadic cancer or some phenotypic traits such as intelligence, which are not usually inherited in the mendelian manner [14,21]. Therefore, the deterministic network suggested by the deductive strategy seems to be only a particular case of a more general, quasi-deterministic network consisting of both deterministic and nondeterministic cellular events [6].

THE GENOTYPE/PHENOTYPE RELATIONSHIP

By definition, the nonbijective relationship between genotype and phenotype denotes that this relationship is neither surjective, because not all elements of a given set of genes possess a counterpart in the set of the phenotypic traits, nor it is injective, because two or more genes may contribute to the emergence of the same phenotypic trait. Also, two or more phenotypic traits may be ascribed to the same gene. Indeed, the same or very similar genotypes may produce distinct phenotypes and, inversely, the same or very similar phenotypes may be associated with distinct patterns of gene expression [3,22–26]. For example, a relatively homogeneous population of normal epithelial cells expressing the same or very similar set of genes undergoes metaplastic transformation when exposed to the conditions existing in the surgical conduits, fistulas, or during chronic inflammation [27,28]. The cells become identical to the other epithelial cell types which are not normally present in a given anatomical region. The intestinal epithelial goblet cells may become normal absorbtive intestinal epithelial cells, gastric parietal cells, or squamous planoepithelial cells [29]. Apparently, the interaction between the extracellular environment and the cellular networks comprising initially identical or very similar sets of actively transcribed genes results in the diversification of phenotype; however, no novel and unknown phenotype arises. Noteworthy, either metaplasia or differentiation of normal cells is a quasi-deterministic process. It is known from both experimental and statistical data that a single nondifferentiated cell may differentiate into a number of well-defined subtypes, as well as how many differentiated cells achieve a stage of a given subtype. This represents a deterministic knowledge about the complex system. However, the final phenotype of the individual nondifferentiated cell cannot be determined in advance, before differentiation is completed, and remains unpredictable (nondeterminism). The unpredictability is caused by the existence of a number of hidden variables within the system (i.e., the variables that neither have been recognized as the ones influencing the system, nor could be controled from outside of the system). Also, a biological process can be deterministic by nature, but the lack of knowledge of the details of this process implies a necessity of stochastic description.

The other example of the nonbijective relationship between genotype and phenotype derives from a study of an in vitro model of colorectal carcinoma, comprising several cell lines resistant to both interferon-α (IFN-α) and all-trans retinoic acid [24,25,30]. A comparative analysis of expression of a number of growth factor-responsive genes in control and treated cells revealed that while all cellular phenotypes were resistant to those factors, patterns of expression of the genes were not identical. A similar variability among the patterns of expression of the retinoid-responsive genes was also observed among several uroepithelial and lung carcinoma-derived cell lines, either sensitive or resistant to all-trans retinoic acid [26,31]. It is possible that this differential expression of growth factor-responsive genes coexisting with resistance of cells to those growth factors reflects uncoupling between a microscopic-scale process such as gene expression and a macroscopic-scale phenomenon such as proliferation.

Given that the patterns of expression of the responsive genes in cells are always known (determinism), no deterministic prediction concerning the phenotypic response can be made on this basis but the probabilistic one. Inversely, given that the phenotypic response to the ligand has been determined experimentally, no consistent pattern of the responsive genes can also be predicted on this basis. Only a probability can be ascribed to the gene pattern associated with resistance or sensitivity. This is because the phenotypic response to the ligands or the relative expression of the responsive genes is, in fact, evaluated statistically at the level of cellular population, and not at the level of a single cell. It is possible to imagine a heterogeneous cellular population comprising both growth factor-resistant and -sensitive cells. Let us assume that the resistant cells express no growth factor-responsive mRNA, and the sensitive cells express a lot of this mRNA under the same conditions. The population would be identified as hormone-resistant or weakly sensitive if the first cell type dominates; however, the pattern of gene expression would reflect the latter subpopulation rather than the first one. Thus, nondeterministic events make the deterministic predictions about the cellular response impossible, and cause that the dynamic cellular network is quasi-deterministic.

Both the issue of the nonbijective relationship between genotype and phenotype and quasi-determinism of the cellular network appears also during interpretation of results obtained by a recombinant DNA technology. This technology is applied to elucidate a role of a gene in phenotype formation at a tacit assumption that cellular network is deterministic, and, therefore, genetic elements can be removed, replaced or inserted without affecting the other parts of the system. The knock-out gene method is used at the assumption that phenotypic trait is inherited in the mendelian manner. It is expected that 25% of all embryos and fetuses are the gene-null homozygotes, which should reveal some phenotypic failure. However, knock-out of every gene does not cause phenotypic abnormalities, nor do those abnormalities appear with a constant and predictable penetrance [32]. Since variability of penetrance is of stochastic nature, i.e., possesses probability, and reflects most likely the stochastic gene activity [6,33], the assumption of mendelian inheritance is an approximation that neglects the coexistence of deterministic and nondeterministic cellular events. The nonbijective relationship between gene expression and phenotypic trait can be observed during the knock-out of genes encoding human retinoic acid receptors. Normal phenotype is present even if the gene is completely deleted because homologous genes of the same superfamily maintain its function [34,35].

In another approach, the gene is transfected to the cells which do not possess it in the genome, nor express both mRNA and active protein. Both selection for cellular clones which re-express the biologically active protein and observation of the phenotypic alterations follow. Gene transfection is a kind of ''directed'' mutation causing phenotypic diversification. Some cells die immediately. Other cells modify rates of growth and proliferation, induce apoptosis, differentiate, or change morphology. In any of this events, no unique phenotypic trait develops. Rather, the ''directed'' mutation modifies the cellular network, restoring one of its previous functions if the gene is incorporated in the right locus. Also, overexpression or down-regulation of the transfected gene produces a number of heterogeneous phenotypes and is a stochastic process. For example, phenotypes resulting from overexpression of Stra 13 gene are not identical with their wild-type counterparts, even if the transfected cells express the same amounts of the active protein [36]. Most likely, overexpression of the gene changes initial conditions within the complex system inducing a number of threshold effects such as activation and inactivation of different molecular pathways, which normally are not influenced by the physiological amounts of the protein. In addition, it appears that only some combinations of the actively transcribed genes corresponding to the unstable stationary states of the cellular network allow the emergence of phenotypes performing biological functions typical of normal cells. The other combinations are lethal or deleterious or inactivate some cellular functions. Consequently, a role of the gene cannot be determined unequivocally by this approach.

CELLULAR CAUSES OF NONBIJECTIVITY

The nonbijective relationship between genotype and phenotype can result from the following features of cells. First, there is no categorical link between genotype and phenotype [3]. The series of genes is a static category, and remains almost the same in each single eucaryotic cell of a multicellular organism. The genotype itself is not subject to selection. The genotype becomes a dynamic category as a part of the cellular network (i.e., when it is activated by the interacting regulatory elements). Then, selection may promote certain privileged combinations of the transcribed genes ''into action.'' Phenotype is a dynamic category. Cellular phenotype is dependent upon both the dynamics of the cellular network and the higher levels of hierarchy [3,6]. It is subject to selection. Now, if the intermediary cellular network was bijective and deterministic, only one phenotype would result from a given genotype. The corresponding gene pattern would only promote the same favorable phenotype. Consequently, the population of cells would remain homogeneous. Instead, two identical sets of genes can easily produce distinct phenotypes producing heterogeneity among cells. Therefore, there is no categorical link between the static genes, which are not subject to selection, and the dynamic cellular network and phenotype, which are subject to selection. This statement is the best exemplified by the observation that the population of initially synchronous and genetically identical cells drifts out of synchrony, producing phenotypes with widely variable cell cycle times within a few cycles. This phenomenon is thought to be caused by random molecular events occurring at the restriction point at the interface of G1 and S phase. At this point, DNA synthesis starts from a random and probabilistic formation of the first pair of DNA replication forks, implying that each cell has a constant probability per unit of time of passing this point and completing its cell cycle [2,37,38]. Again, a stochastic process underlies phenotypic diversification of the genetically identical cells. Second, no single gene or set of cell type-specific genes can entirely account for a given cellular phenotype [5–7,23,39,40]. Experimental data point out that cellular phenomena including phenotype formation are usually accompanied by expression of a large number of genes organized in a hierarchical network [2,4,20,23]. While the cell types in a multicellular organism express different genes from the same genome, surprisingly only few differences in protein content distinguish one cell type from the other [4,6,7]. The fate of a cell appears to be determined in time gradually, not just by the quantities of proteins expressed. It is dependent on

expression of a master gene that possesses the characteristics of a bifurcation point gene [6]. The master genes develop their action through a number of master gene regulatory proteins that interact with enhancer elements of other genes organized in the temporal and spatial hierarchy [41]. Consequently, a hierarchy of genes unique for a given cell type is controlled by a few elements and is subject to reorganization towards a novel hierarchy of genes. For example, transformation of fibroblast into myoblast phenotype can be achieved due to transfection of a single master gene [42]. In addition, the Kauffman model of the interactive gene network suggests that genes involved in phenotype formation are expressed continuously in a cyclic manner [23]. Therefore, the number of phenotypes arising is much lower than the number of genes involved in the process. Cellular phenotype remains stable as long as the modifications of gene expression occur within the underlying cycle, no matter what combination of genes are on or off at that moment. The model points out that phenotype formation is not a result of expression of a single gene or of a group of the selected genes. It is a result of interactions within the hierarchical gene network. Now, if the network were deterministic, a continuous change of patterns of gene expression would lead to the continuous emergence of novel phenotypic traits. Yet, phenotypic traits emerge in a discrete manner. The ultimate cell types are sharply distinct, and no intermediary phenotypes exist that would represent a mixture of phenotypic traits typical of the final phenotypes. Although cell type-specific genes do exist, they represent a minority of the actively transcribed genes in a cell and encode proteins needed only in this type of cells or contribute to development of some unique, tissue-specific traits [5–7,39,40]. In addition, tissue-specific traits can also develop as a result of cooperation between a number of nonspecific molecular elements. For instance, the secretion of milk protein, a unique phenotype of the mammary gland, is controlled by a number of hormones and hormone receptors such as prolactin and its receptor, which are not unique to this tissue.

NONLINEARITY, COMPLEXITY, AND FRACTAL STRUCTURE

The nonbijective relationship between genotype and phenotype implies the existence of nonlinearity or complexity in the cellular network [8,9,24]. Nonlinearity denotes that a constant increment of the independent variable does not cause the proportional increment of the function value or the outcome. A plot reflecting the relationship between the input and output would not be a linear curve. As a result of nonlinearity, the cellular network has much more complicated intrinsic structure than that of the linear one, and, moreover, the network goes through a number of chaotic states. Furthermore, complexity requires the existence of several yet undefined levels of cellular regulation that are very sensitive to extra- or intracellular alterations [43]. This results, most likely, from the coexistence of complexity and deterministic chaos (Kolmogorov-Arnold-Moser theorem) within the molecular and energetistic structure of cells [6,7,12]. Consequently, either metabolism [44–46], cytoskeletal patterns [47], calcium sequestration [48], tissue morphological patterns in normal or tumor tissues [6,49–53], or tissue growth [54,55] possess fractal dimension. The fractal dimension reflects the intriguing fact that cells, in spite of the daily experience in the three-dimensional euclidean reality, which approximates geometric reality, are intermediate between two-dimensional surfaces and three-dimensional objects, and possess a fractal structure. By definition, the fractal structure has the algebraic equivalent of the general form

$$y = ax^b$$

called power law, where b is the non-integer (i.e., fractal) dimension, a is a coefficient, x is a distance, and y is the number of scale-invariant objects [56]. The fractal structure implies that the real time of cellular processes is a part of the dynamic space–time geometry. This denotes that a biological process occurs in the fractal space that influences its own structure in the active manner. The projection of dynamics of the process onto the three-dimensional space, which occurs during the quantitative follow-up of the process in time, implies uncertainty. This imposes limits on the experimental access to such system, on the accuracy of the molecular models, and, in particular, on the interpretation of the relationship between genotype and normal or malignant phenotype.

QUASI-DETERMINISM, SELF-ORGANIZATION, AND GENETIC MODEL OF TUMORIGENESIS

According to the genetic model of tumorigenesis, gene mutation is the cause of cancer. Tumor progression is driven by a sequence of mutations in a limited number of genes (causality). In this model, gene mutations, which are ordered in time, are related bijectively to the emerging phenotypic traits typical of malignant cells (determinism) [19,57,58]. For example, tumor cells gain metastatic potential because the p53 gene mutates [59]. Interestingly, no mutations proposed to drive tumor progression are lethal to cancer cells, nor are lethal mutations occuring after the failure of the DNA repair gene system [60]. Finally, a competition between cancer cells eliminates many of them, producing a monoclonal population of cancer cells with relatively similar distribution of genetic defects (natural selection) [61]. Formation of metastases seems to be both unavoidable and predictable because cancer cells that have reached this stage of progression have both goal-oriented structure and apropriate

gene defects, i.e., mutations in p53, DCC, and MCC gene (teleology) [19,58,62,63]. The genetic model of tumorigenesis neglects, however, the phenomenological nature of the definition of tumor suppressor genes and oncogenes, their tissue relativity [6,64–66], oncogenic potential of randomly mutated proteins [67–70], the partial dependence of tumor cells on their neuroimmunohormonal and stromal environment [71], and epigenetic events contributing to tumorigenesis [22,72].

The concept of the nonbijective, nonlinear, complex, and quasi-deterministic cellular network offers alternative interpretation of tumorigenesis-related events, where both epigenetic and genetic events coexist, are not mutually exclusive and contribute to the process equally. Whereas the emergence of normal cellular phenotype involves expression of a number of genes, which is ordered in time, the emergence of malignant phenotype seems to comprise additional events. Those events occur most likely at the thermodynamic level [73].

Either normal or transformed cells interact with their physical and chemical environment [22,72], a fact best exemplified by the Hormesis phenomenon in which the interaction between physical environment (i.e., in this case low-intensity ionizing radiation) and biological system resulted in a nonlinear effect [74–76]. Interestingly, the linear interpolation of the results of the experiments where high-intensity radiation was applied suggested that low-intensity radiation must also have a harmful rather than beneficial influence. The cellular network remains sensitive to the environmental influences because the interaction modifies patterns of gene expression and induces differentiation [77,78]. The interaction between cells and their physical, chemical, and biological environment evokes at least two kinds of effects. Cells respond in the algorithmic manner, inducing gene expression, proliferation, differentiation, and apoptosis, or they die immediately. Simultaneously, those interactions cause that macromolecules (i.e., DNA, mRNA, and proteins) undergo thermodynamic fluctuation [15,79–81]. The fluctuations occur when the macromolecules undergo the chaotic oscillations caused by the action of a physical force whose average value in time is zero. Most frequently, the fluctuations disappear if the stimulus ceases. Then, the system has a chance to return to the former thermodynamic equillibrium; howver, probability that it would be the original state or conformation is very low. More likely, if the fluctuations persist, they can change the structure of the entire system. The system moves toward a novel thermodynamic equilibrium. Since energy neccessary for this transformation and for maintaining the emerging structural defects derives from the nearest, intracellular environment, re-self-organization of the entire cellular network follows, not just gene structure alone. Self-organization can only be expected in a nonbijective, and, therefore, nonlinear and complex system because it requires a number of intrinsic feedbacks. Those so-called negative feedbacks self-constrain any hierarchical structure such as atom, simple molecule, macromolecule, a chain of enzymatic reactions and metabolic cycles, signaling pathway, cell, tissue, organ, organism, ecosystem, or society. The more feedbacks exist in the complex system, the more complex it is; however, the inverse relationship is also possible. Furthermore, the process of re-self-organization is of autocatalytic nature, (i.e., each additional fluctuation of energy in the complex cellular system keeps the system further away from the original conformations of macromolecules, and a critical number of such fluctuations can result in the rapid emergence of persistent structural defects). It is, therefore, likely that so-called mutator phenotype of cancer cells can develop without underlying mutations in the DNA repair gene system of mitotic checkpoint gene system. Self-organization is not an utterly random process, nor is it a purely deterministic one. On one hand, self-organization follows a set of universal rules which cause that a number of cellular phenomena possess fractal structure (see above), L-amino acids, rather than D-amino acids, are incorporated during protein synthesis, that the genetic code is degenerated [2,4,5], and that the number of metabolic pathways or cell phenotypes for a single cell to choose from, is very limited [23]. On the other hand, even a relatively simple deterministic molecular system of a few gene regulatory proteins such as this present in bacteriphage lambda generates during the bacteriophage life cycle a sophisticated pattern of dynamic behavior, which also involves probabilistic effects such as recognition of the current status of the host cell [82]. Recently, it was suggested that tumorigenesis is an entirely deterministic chaotic process [83,84]. It is certainly possible that deterministic chaos underlies some relatively simple cellular phenomena such as cardiac tachyarrhythmias. It is possible that those phenomena could be controlled by introducing small critically timed perturbations [85]. However, tumorigenesis seems to be driven by the interplay between complexity, deterministic chaos, self-organization, and natural selection [6]. This means that tumorigenesis possesses much more complicated dynamics than dynamics of deterministic chaos. Furthermore, cells undergoing re-self-organization remain far from any stable stationary state. The suggestion that cancer cells remain within an attractor seems to be premature. By definition, attractor is a fragment of the space where the process occurs, and where no further evolution of the system is possible at all. It is unlikely that cancer cells achieve such attractor immediately after malignant transformation or while progressing.

It appears that neither gene mutations nor natural selection is able to generate novel cellular phenotypes in a bijective manner. Random gene mutations, which are material traits of the thermodynamic fluctuations of energy, enforced by alterations of both DNA methylation and enzyme activity can only modify the pre-existing intracellular organization or destroy it utterly [86,87]. These events eliminate some of the feedbacks that normally self-constrain the cellular network. It leads to decomplexification of the normal cellular network, development of a novel hierarchy of genes, and self-organization of the derivative and, also, quasi-deterministic, cellular network. Decomplexification should be expected to produce a lower fractal dimension, more differences in protein types between normal and cancer cells [88], uncoupling between microscopic-scale phenomena such as gene expression and macroscopic-scale phenomena such as proliferation, and instability of the network [6,24,25].

As a result of instability, any environmental or intracellular impulse can initiate one of the algorithmic cellular responses, such as proliferation, incomplete differentiation, detachment, or infiltration, resulting in the breakup of tissue organization—well-known features of malignant tumors. Since the network is quasi-deterministic, a stable phenotype of malignant cell can emerge due to the action of the equivalent and alternative dynamic cellular events which are not ordered in time, nor in fractal space of cellular events [73,89]. While a cause of malignant transformation and tumorigenesis certainly exists, the link between this cause and phenotype cannot be identified unequivocally. This link can only be described in terms of the probabilistic associations [6]. The existence of the sequence of genetic defects, which would be ordered in time, and related bijectively to the emergence of malignant phenotypic traits is unlikely in the quasi-deterministic cellular network. Such sequence results most likely from neglecting the influence of natural selection. Natural selection denotes that the extracellular environment selects against the less favorable cells promoting phenotypes that continue to succeed during competition, and eliminating those that are deleterious. Because the physical limits of a complex tissue system, where a single cell exists, cannot be easily defined, natural selection can be considered as a part of the intrasystemic forces engaged in the process of self-organization. Although the alternative pathways of tumorigenesis has been introduced to the genetic model [90], they underlie only special morphological and genetic forms of colon cancer. The predefined sequence of gene defects remains the cornerstone of the genetic model.

PRACTICAL INFERENCES

The presented concept of the dynamic cellular network possesses also practical consequences. First, fractal analysis can be applied to design an objective and quantitative system of tumor grading. Instead of subjective evaluation performed by a pathologist, a coefficient of linear regression corresponding to the fractal dimension can be calculated on the basis of distribution of morphological patterns such as gland-like structures or bundles [56]. In this way, different sections within the tumor can be characterized by a series of fractal dimensions. Low values of the fractal dimension point out that decomplexification of the population of tumor cells is advanced, and a risk of metastases is high. Second, decreasing values of the fractal dimensions in normal-appearing tissue can suggest the increasing risk of tumorigenesis. This parameter could be applied to monitor postoperatively, e.g., colorectal cancer patients. It must be emphasized, however, that experimental studies are necessary to investigate how this parameter changes during different stages of tumor progression. Third, the fractal dimension can be applied to follow up biochemical and molecular changes associated with tumor formation or the influence of drugs on tumor growth and geometry. It can also be expected that fractal analysis of tumor growth will demonstrate a chaotic periodicity of progression and metastasizing in some types of cancer. In such case, the increased self-order of cancer cells can be achieved by modification of a single biochemical parameter controlling cellular hierarchy [84]. Finally, the concept suggests that biomarkers can monitor tumor progression in quasi-deterministic rather than deterministic manner. In other words, evolution of the process can be described in terms of associations and probabilities rather than absolute certainty.

A discrepancy between the deductive strategy of molecular reductionism and the experimental findings outlines a novel perspective in cellular biology. From this perspective, physics and biology belong to the same science of complexity that bridges structure and dynamics. Without invalidating molecular findings, complexity offers an alternative, quasi-deterministic interpretation of cellular events underlying phenotype formation and tumorigenesis. The following points need to be emphasized as crucial consequences of the available experimental data: the relationship between genotype and phenotype reflects nonbijective interaction between static and dynamic categories [6,24–26,30,31] leading to nonlinearity of the intra- and intercellular interactions [6,8,9,24], nonlinearity implies the existence of the fractal structure of space of cellular events [6,44–55]. Those features support an holistic rather than a reductionist approach to cellular and cancer biology. Because of the possible ho-

listic nature of cellular phenomena, they must now be investigated at different levels of the hierarchical structure of a cell in a complementary manner.

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